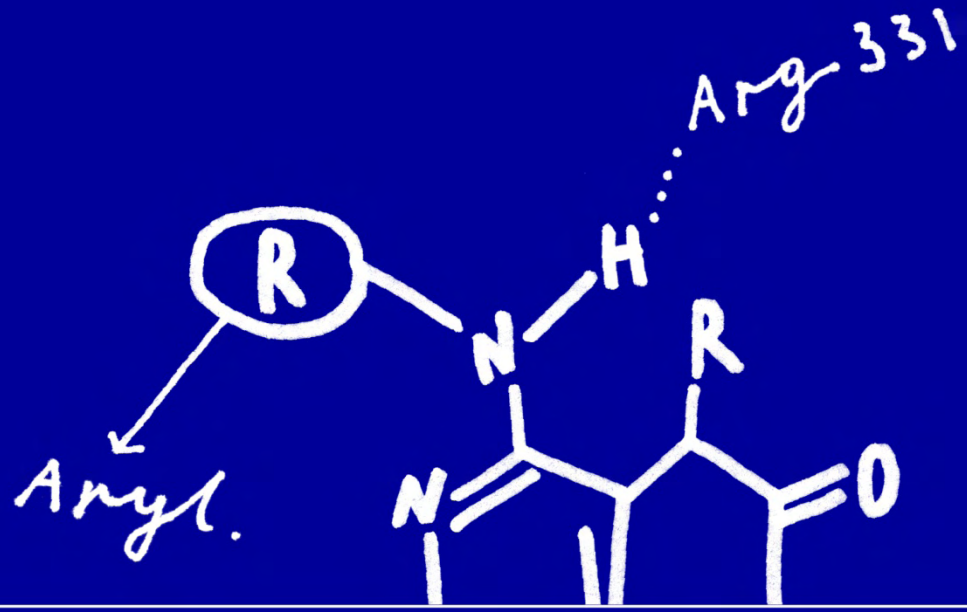
evotec

'RESEARCH NEVER STOPS'

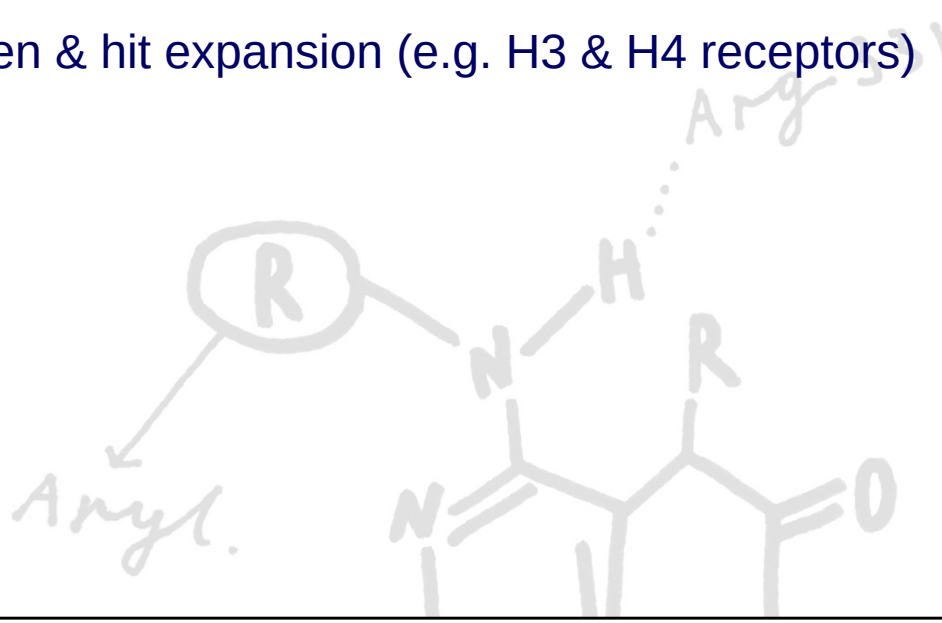
Building innovative drug discovery alliances

Novel histamine GPCR family antagonists by fragment screening and molecular modelling



Outline of Presentation

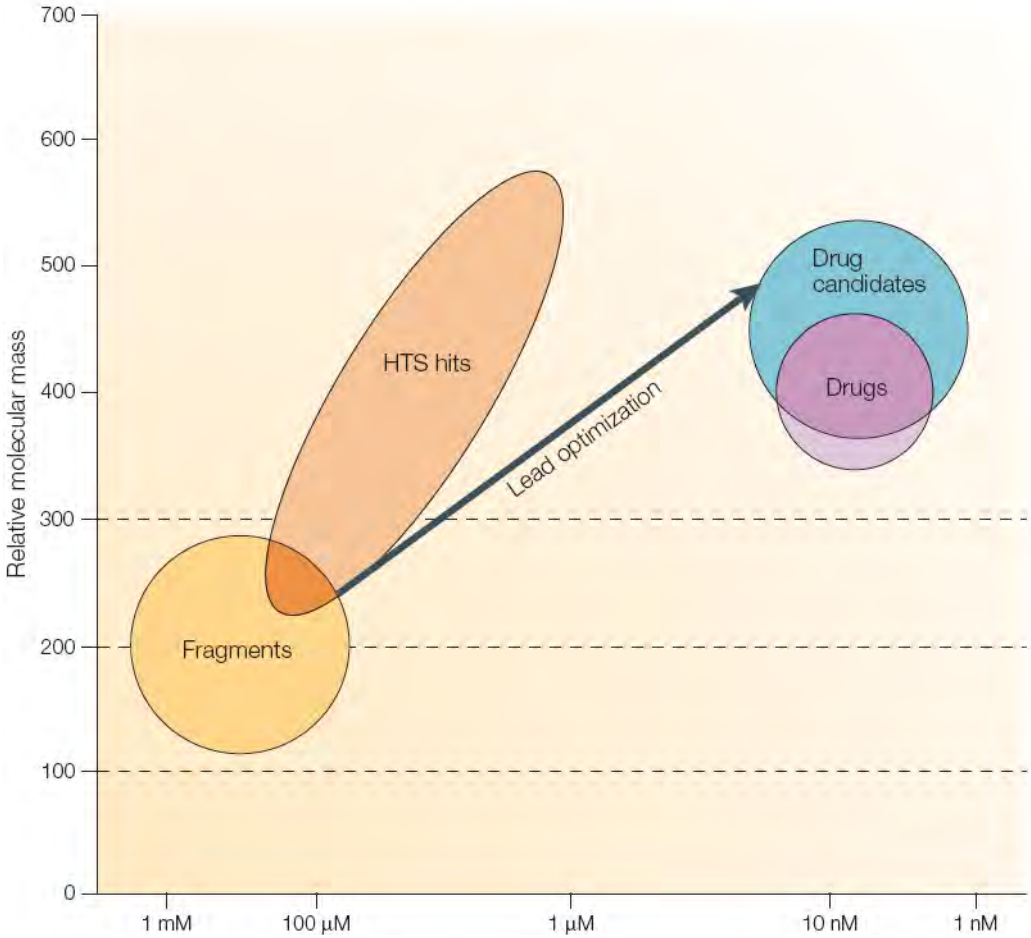
- Fragment screening and fragment evolution
- Intro to hierarchical GPCR modeling (e.g. Bradykinin-1)
- GPCR fragment screen & hit expansion (e.g. H3 & H4 receptors)



Defining a fragment

Low molecular weight weakly active hit compounds

- ‘Rule of 3’ (e.g. <300MW, <3 clogP)
- Approach is best suited to targets for which protein X-ray structures can be readily obtained
  - Rapid iterations by structure based design
- ‘Build in’ drug-like ADMET properties
  - Limit undesirable, excess features and ensure good solubility etc.
- Excellent coverage of chemical diversity
  - Novel start points with space for optimisation
  - Ligand efficiency



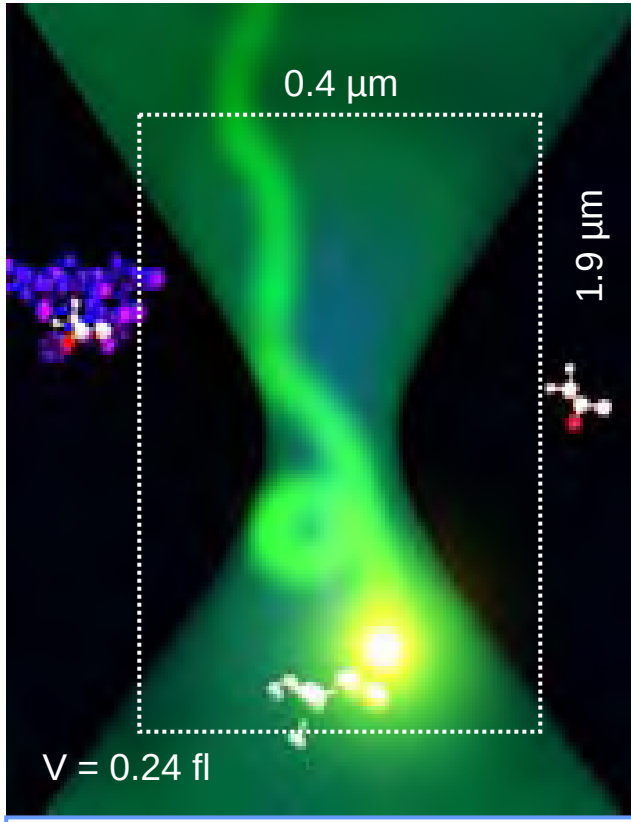
D.C. Rees et al., Nat. Rev. Drug Discov., 2004, 3, 660-672.

Kerseru & Makara. Nat. Rev. Drug Discov., 2009, 8, 203-212

Ultra-sensitive biochemical screening technology: Fluorescence Correlation Spectroscopy (FCS+plus)

Balancing sensitivity and throughput

- Key to fragment screening is to balance sensitivity with throughput
- FCS+*plus* screening technology more sensitive than traditional biochemical screening methods
  - Detection at the single molecule level through confocal laser technology
  - Multiple readout parameters (translational/ rotational diffusion, fluorescence intensity etc.) allows selection of the optimum readout
  - Clearly distinguishes between noise and signal Reduces the number of false positives
- Ultra high throughput allows rapid screening of fragments (>20,000 fragments)



Occupancy of confocal volume = 1.5 molecules at 10 nM

Law et al (2009). The Multiple Roles of Computational Chemistry in Fragment-Based Drug Design. JCAMD,23(8);459-473.

Fragment Development

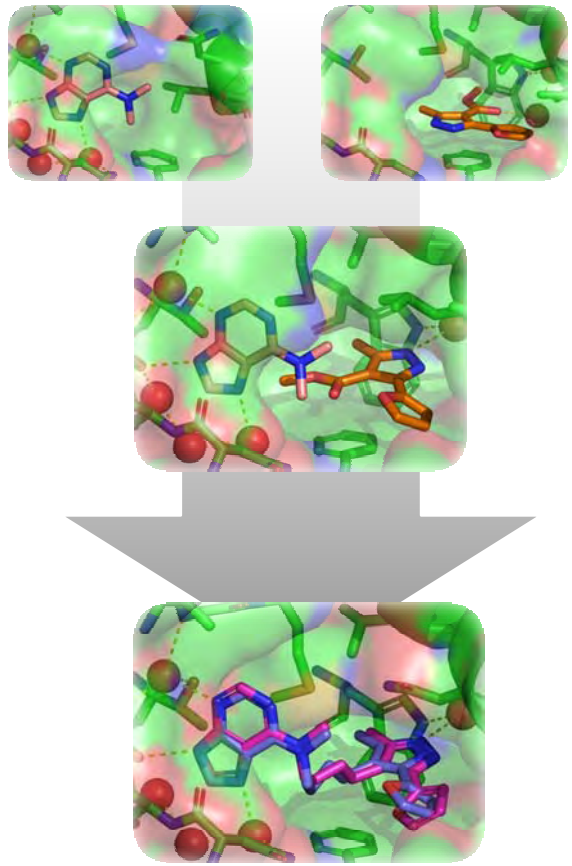
Fragment evolution, linking and hybridization

Fragment Evolution →
Identify most promising fragment
- Ligand efficiency
- Confidence in binding mode
- Chemical expansion vector
- Synthetic tractability
- Secure anchoring points

SAR by nearest neighbour →
Search for commercial analogues
- (14M library at Evotec)
- Purchase and test

Fragment Linking →
ID of adjacent fragments
- Adjacent fragments can be linked
- Maintenance of interactions and poses and reduction of strain energies

Hybrid with existing leads →
3D Overlay with existing leads
- Design by visual inspection
- Scaffold hopping and linker search tools



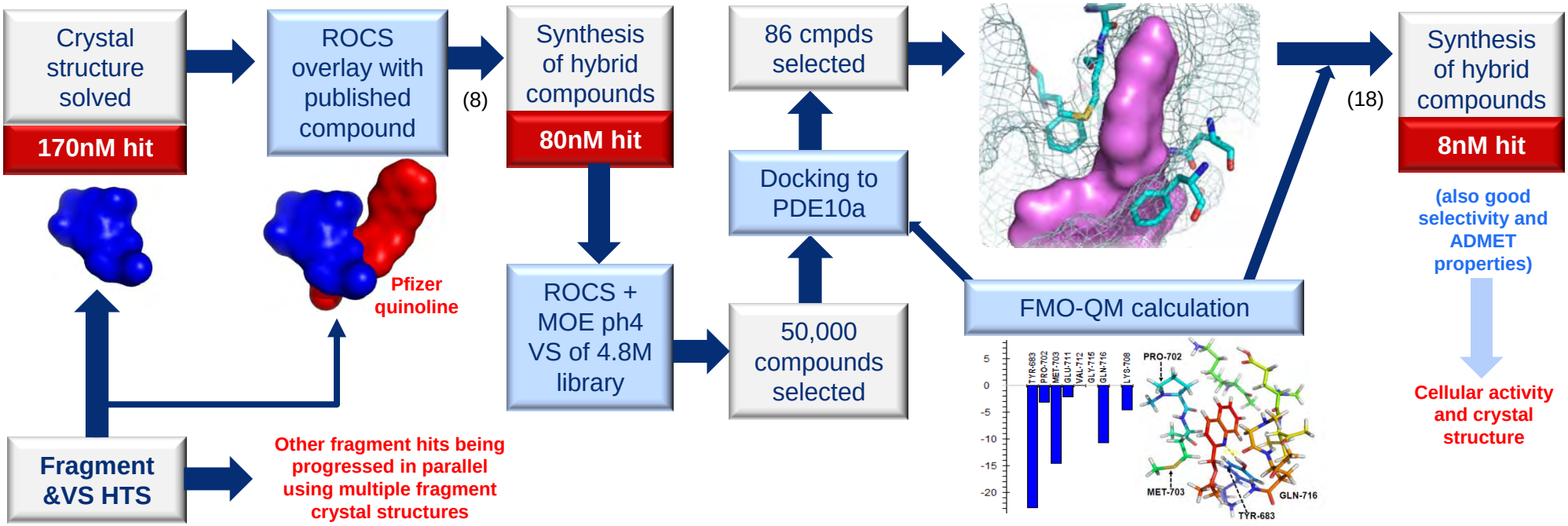
Hsp90 fragment linking example[1]

[1]Barker *et al.* (2010). Discovery of a Novel Hsp90 Inhibitor by Fragment Linking. *ChemMedChem* DOI: 10.1002/cmdc.201000219

An example of comp. chem. driven expansion of a fragment hit

PDE10a case study

- Fragment structures and comp. chem. used in to drive accelerated fragment-to-lead



Law et al (2009). The Multiple Roles of Computational Chemistry in Fragment-Based Drug Design. JCAMD,23(8);459-473.

Fragment based drug discovery and GPCRs

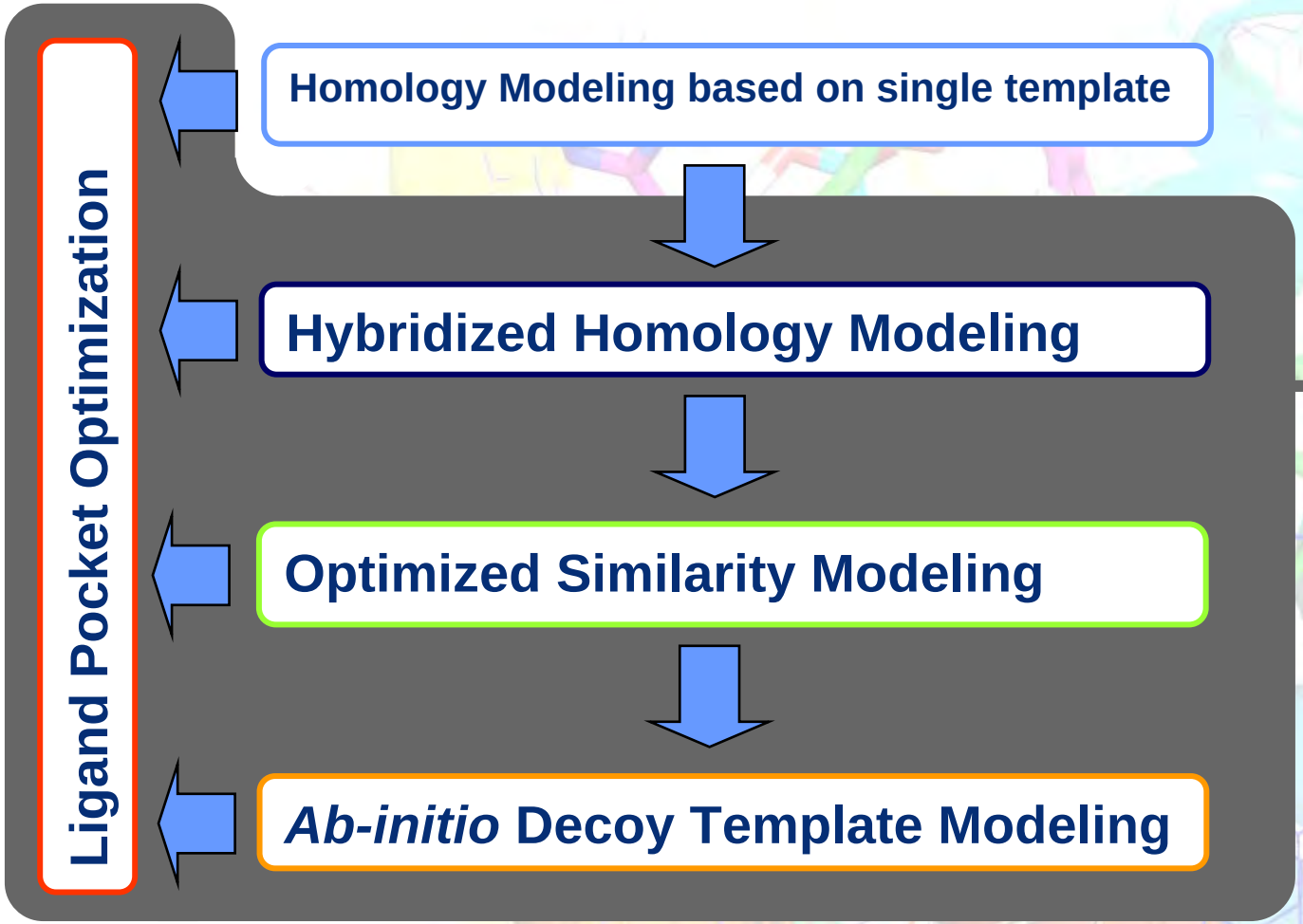
Reasons to screen fragments on GPCRs

- Privileged sub-structures/fragments have been identified in ligands for a wide variety of different GPCRs
- Assess drugability of target (orphan GPCRs?)
- Novelty
- Endogenous ligands and lead-like compounds for biogenic amine receptors have structures similar to fragments

- Molecular modeling allows fragment binding to be rationalized and allows for both structure and ligand based hit expansion
- The emerging structural data for GPCRs makes structure-based design, at this level, possible for GPCRs
- We have established hierarchical GPCR modeling protocols

Evotec's Hierarchical GPCR Modelling Software

An overview



Evotec GPCR Modeling Scheme

- With only 4 GPCR xstal structures, and >1000 GPCRs, a predictive model via homology is unlikely.
- The model is automatically assessed for quality at each level
- It is passed to the next layer of complexity if it fails
- Each stage involves proprietary code

- Each tier of the modeling process assesses the need for the next stage

GPCR specific seq. alignment matrix

Helical rotation alignment

Addition of SDM data

MD kink detection & formation

Helix tilt optimization

Loop remodeling

MC side-chain rotamer library sim.

Homology Modeling based on single template

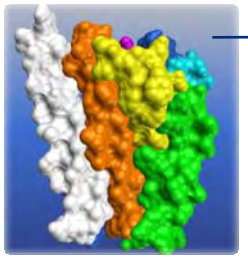
Hybridized Homology Modeling

Optimized Similarity Modeling

Ab-initio Decoy Template Modeling

Hierarchical modeling assessment

Ligand Pocket Optimization



Protein packing & complementary score

Fit to sequence alignment

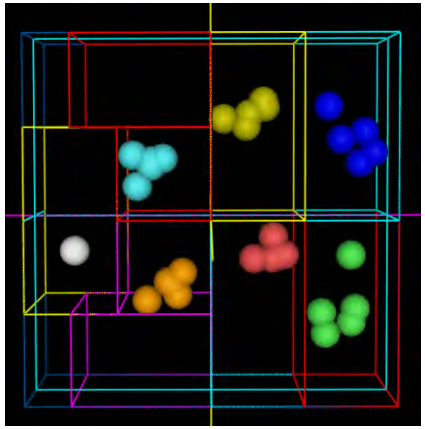
Individual optimization routine scoring

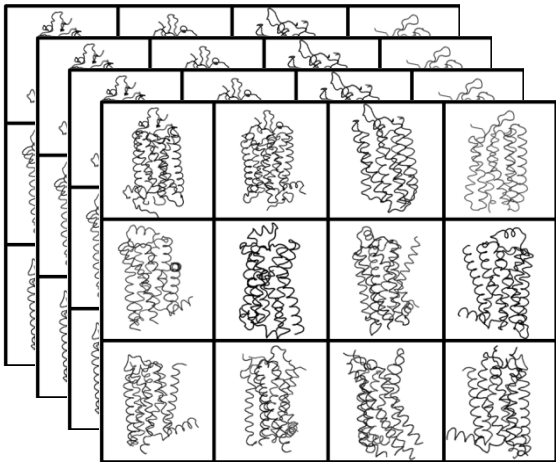
Fit to SAR and SDM data

Ab-initio level of GPCR modeling

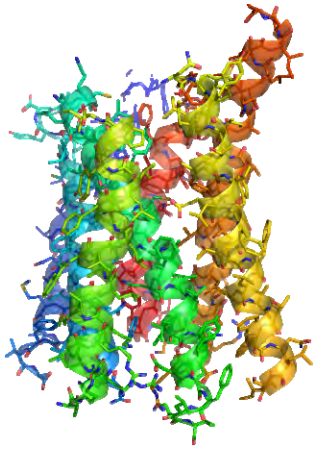
Getting structures most distant from known GPCR structures

Ab-initio Decoy Template Modelling





- Helical rotation alignment
- MD kink detection & formation
- Helix tilt optimization
- Loop remodeling
- MD optimiz. of binding pocket
- Addition of SDM data
- MC sidechain rotamer library sim.



MC & MD optimized *ab-initio* model

- Template library of 5,700 *ab-initio* constructed
- GPCR model start points are selected
- Optimized by the MC & MD routines

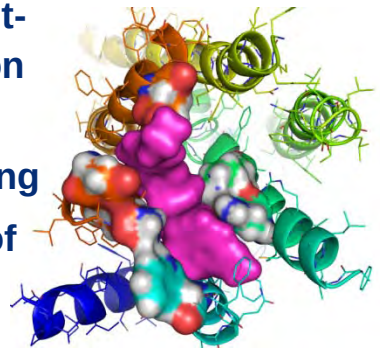
- Enables modeling of previously inaccessible GPCR structures – e.g. distant relations, like Orexin, and some orphan GPCRs

Applications of GPCR Modelling

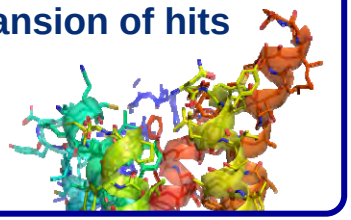
Some examples of the application of the methods

- **Comp. Chem. group has produced GPCR models for many of Evotec's GPCR projects**
- **GPCR model can be used in many stages of drug design:**
  - Virtual screening
  - Rational optimisation of HTS hits
  - Hit-to-lead and Lead optimization support

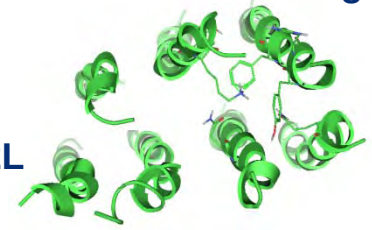
- **Bradykinin 1 – use in hit-to-lead/lead-optimization**
- **Required optimized similarity tier of modeling**
- **Enabled development of sub-nM, rat & human equipotent compounds**



- **Histamine H3/H4 - models used to guide H3 H2L + LO**
- **Fragment screen of H3 + H4 conducted**
- **OS model used in hit expansion of hits**
- **Very high enrichment**
- **Double-digit nM hits**



- **Orexin – homology used to suggest site-directed mutagenesis**
- **SDM data used as part of ab-initio modeling of OX1 and OX2**
- **Selectivity explained**
- **Being used to guide H2L**



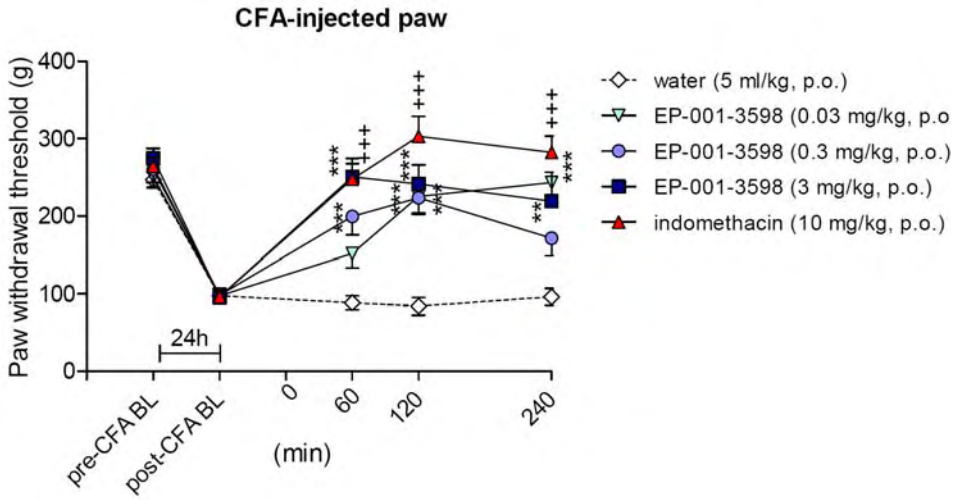
- Also CB1 (Agonist & antagonist) example, recent Alchemia MCH-1 example, and others!

Bradykinin 1 (B1)

An example of GPCR modeling used for a complex GPCR structure

- Fast-follower ***incorporating receptor modelling*** to identify optimal chemistry starting points
- Additional 250K HTS performed as backup giving supporting, novel chemical equity

	EP-001-3598
hB1 IC_{50} (nM)	**0.7**
rB1 IC_{50} (nM)	**0.5**

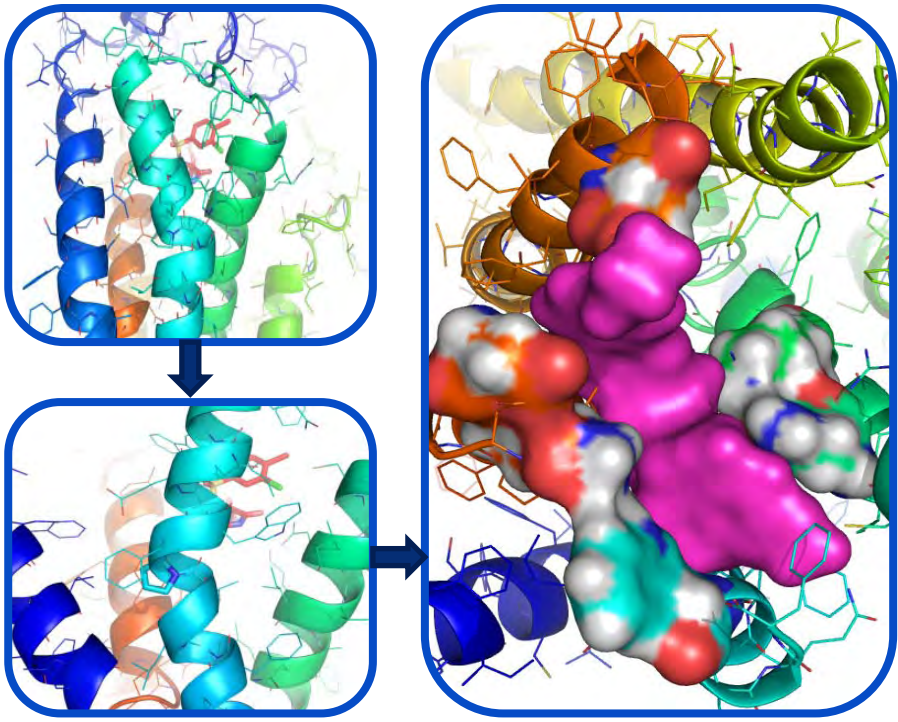


- EP-001-3598 exhibits oral efficacy in rat CFA model of inflammatory pain (MED 0.06 mg/kg po)
- Hit-to-lead & lead optimisation used ***GPCR models to drive SAR***

Summary of B1 Modeling

TM2 and TM6 required optimized similarity modeling

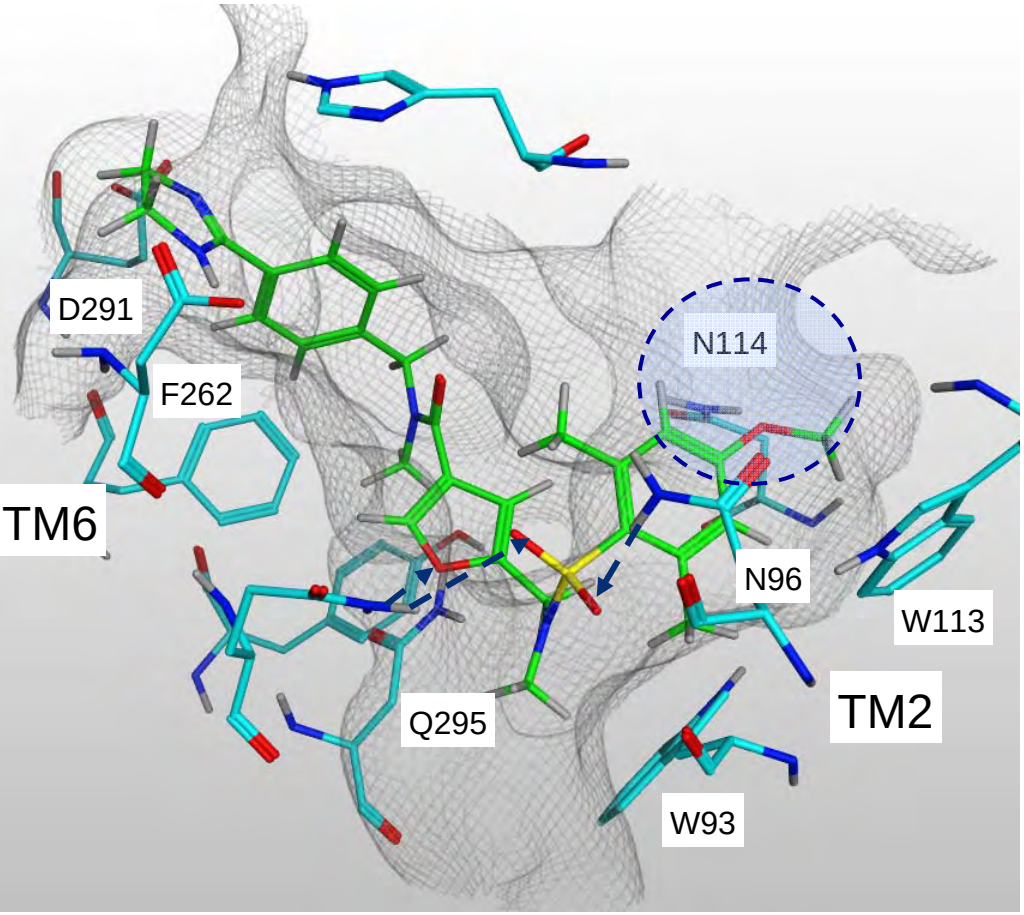
- Modeling of B1 - supported H2L and LO
- Simple homology modeling was unable to produce a model that could explain our SAR (even though it did explain some published B1 SAR)
- Due to unavoidable error in structure – i.e. incorrect proline driven kink in TM2
- Optimized similarity modeling corrected the TM2 error, also TM6 rotation different to homology model
- Helped to drive LO of potent B1 antagonists
- Process repeated for rat B1 and was able to drive the production of compounds equipotent in rat & human – a very important milestone in the project

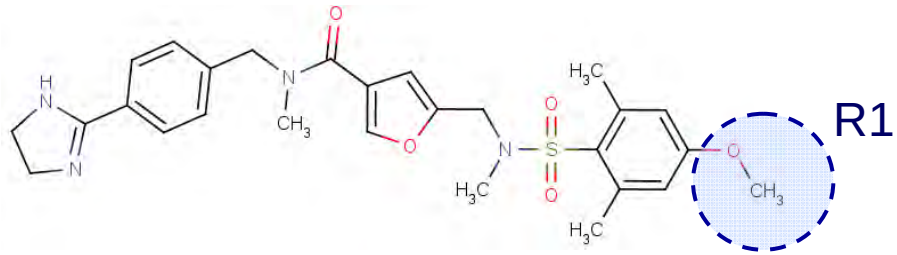


Pal et al. (2010). Prediction of the 3D Structure of Human Bradykinin B1 Receptor and SAR exploration of known human B1 receptor antagonists. J. Med. Chem. (Submitted)

Evotec furan sulfonamide compound

Fast-follower series in the B1 pocket – SAR guidance





EP-001-3264 hB1 = 0.5 nM

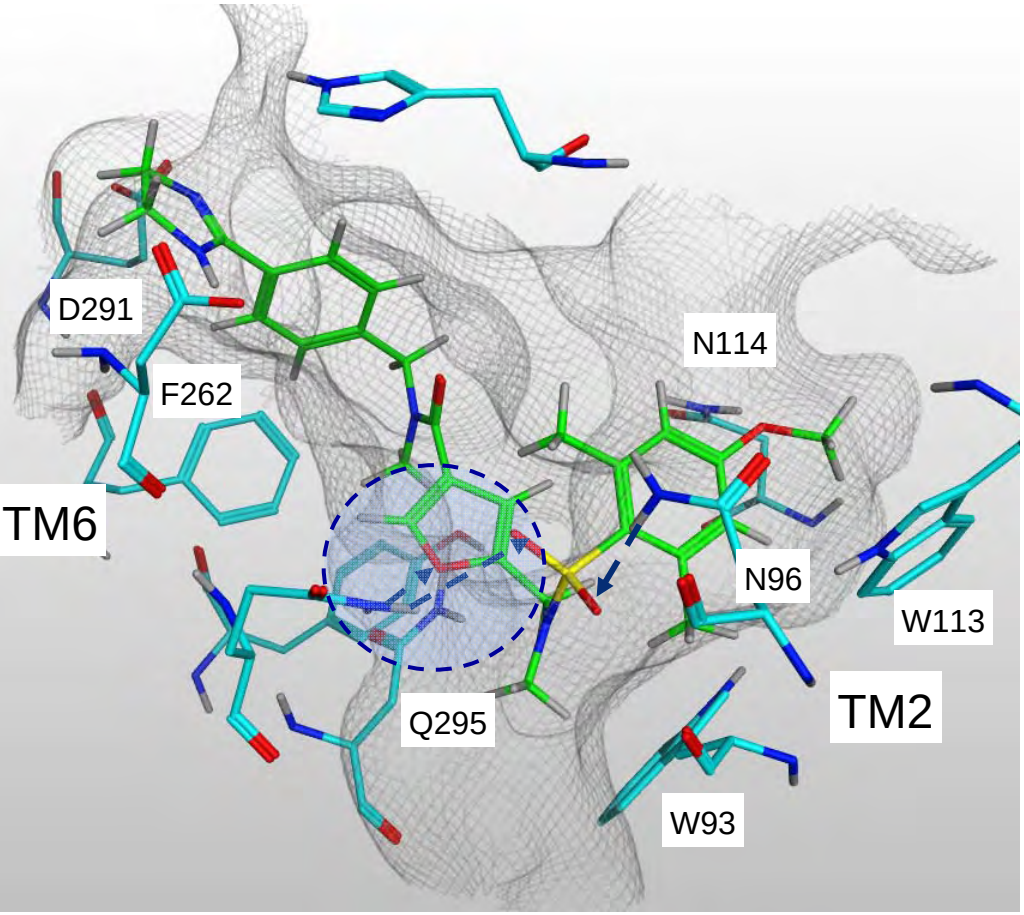
Many features of this and the lead series were guided by the model – e.g.

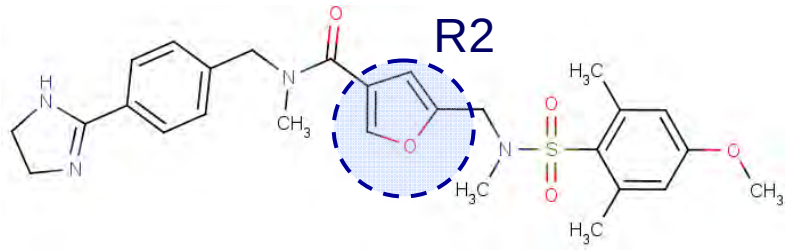
- **Methoxy group H-bonding to N114 is crucial**

R1	hB1 (IC50 nM)	rB1 (IC50 nM)
-OMe	0.5	117
-Br	176	-
-H	11316	-

Evotec furan sulfonamide compound

Fast-follower series in the B1 pocket – SAR guidance

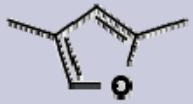
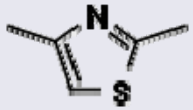




EP-001-3264 hB1 = 0.5 nM

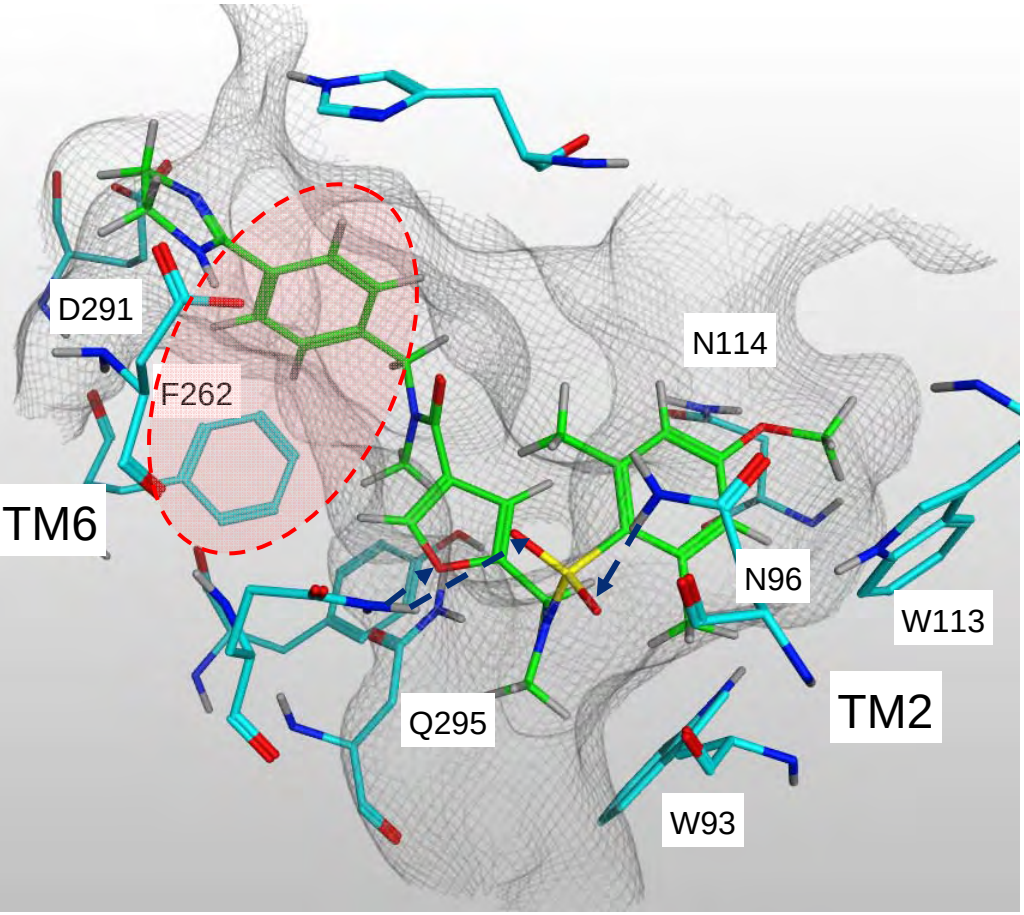
Many features of this and the lead series were guided by the model – e.g.

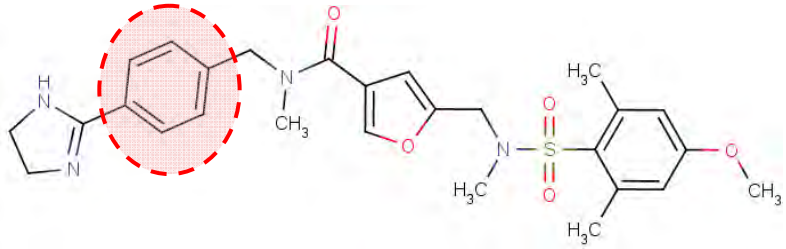
- **Methoxy group H-bonding to N114 is crucial**
- **Furan Oxygen H-bonding to Q295 crucial**

R2	hB1 (IC50 nM)	rB1 (IC50 nM)
	0.5	117
	244	1562

Evotec furan sulfonamide compound

Fast-follower series in the B1 pocket – SAR guidance





EP-001-3264 hB1 = 0.5 nM

Many features of this and the lead series were guided by the model – e.g.

- **Methoxy group H-bonding to N114 is crucial**
- **Furan Oxygen H-bonding to Q295 crucial**
- **F262 (TM6) stacking possible only by the OS/MC procedures (Homology modelling would have oriented TM6 towards the membrane side).**

Outline of Presentation

- Fragment screening and fragment evolution
- Intro to hierarchical GPCR modeling (e.g. Bradykinin-1)
- **GPCR fragment screen & hit expansion (e.g. H3 & H4 receptors)**

Histamine H3 Receptor

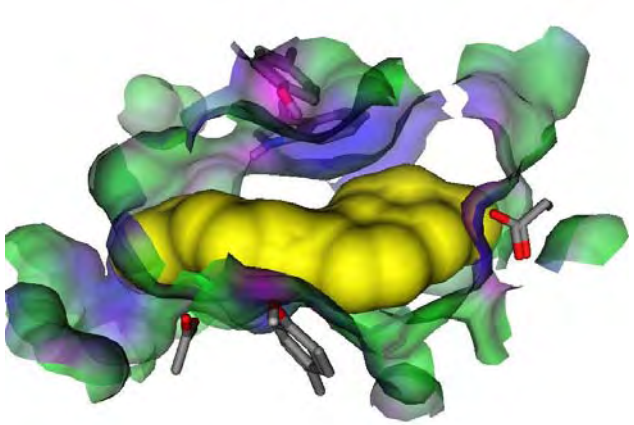
Some basic facts about H3

- H3 receptor identified as pre-synaptic receptor
- As auto-receptor H3R regulates the release of histamine
- As hetero-receptor H3R regulates the release of other neurotransmitters, including Ach, 5HT, DA, NE
- H3 receptors are mainly localized in brain and peripheral nervous system
- Indications discussed for H3 Receptor modulating substances include:
  - Cognitive disorders
  - Narcolepsy / fatique
  - Insomnia
  - Nociception
  - Neuralgia (neuropathic pain)
  - Obesity

Histamine H3 clinical candidate discovery

Indication: Cognition and Sleeping Disorder

- 250K HTS of Evotec compound collection performed, counter screen against H1, H2 and H4 identified H3 selective clusters
- Virtual screen with optimized H3 computational models identified additional SAR which was confirmed in secondary models
- GPCR modelling used throughout H2L and LO
- Strategy delivered two potent (sub-nM *in vitro*) compound classes with ***in vivo* efficacy**
- Currently a pre-clinical development candidate – advancing to FIM (First in Man)



- Efficacy data (SD Rat)

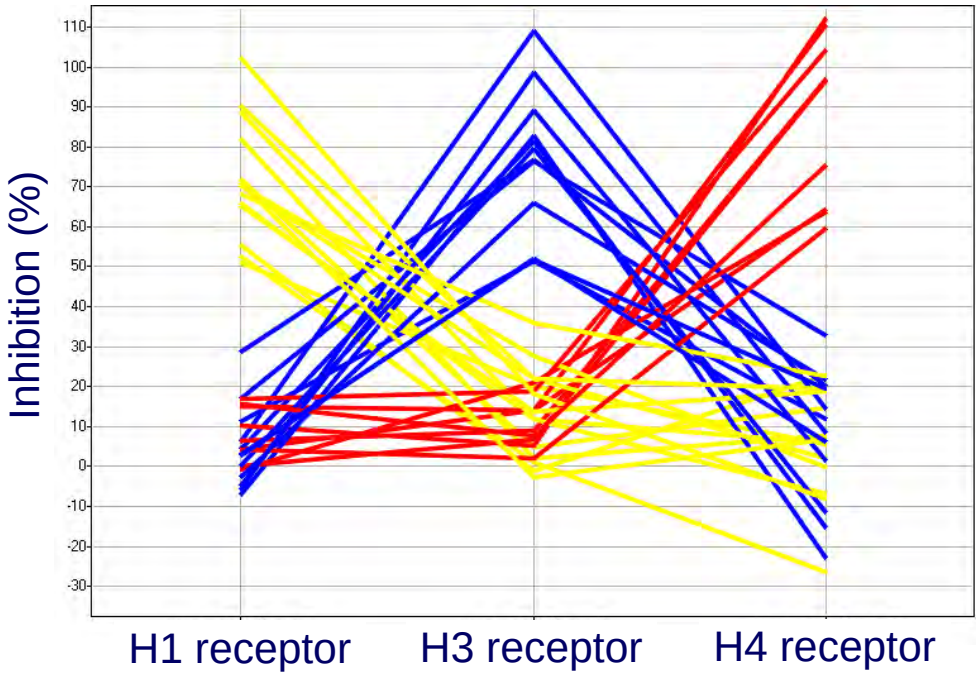
  3 mg/kg p.o.

  - Dipsogenia full reversal
  - Time-course dipsogenia
  - Microdialysis (HA, ACh)
  - Passive Avoidance
  - EEG awakening effects

Fragment screen on histamine receptors

Key information

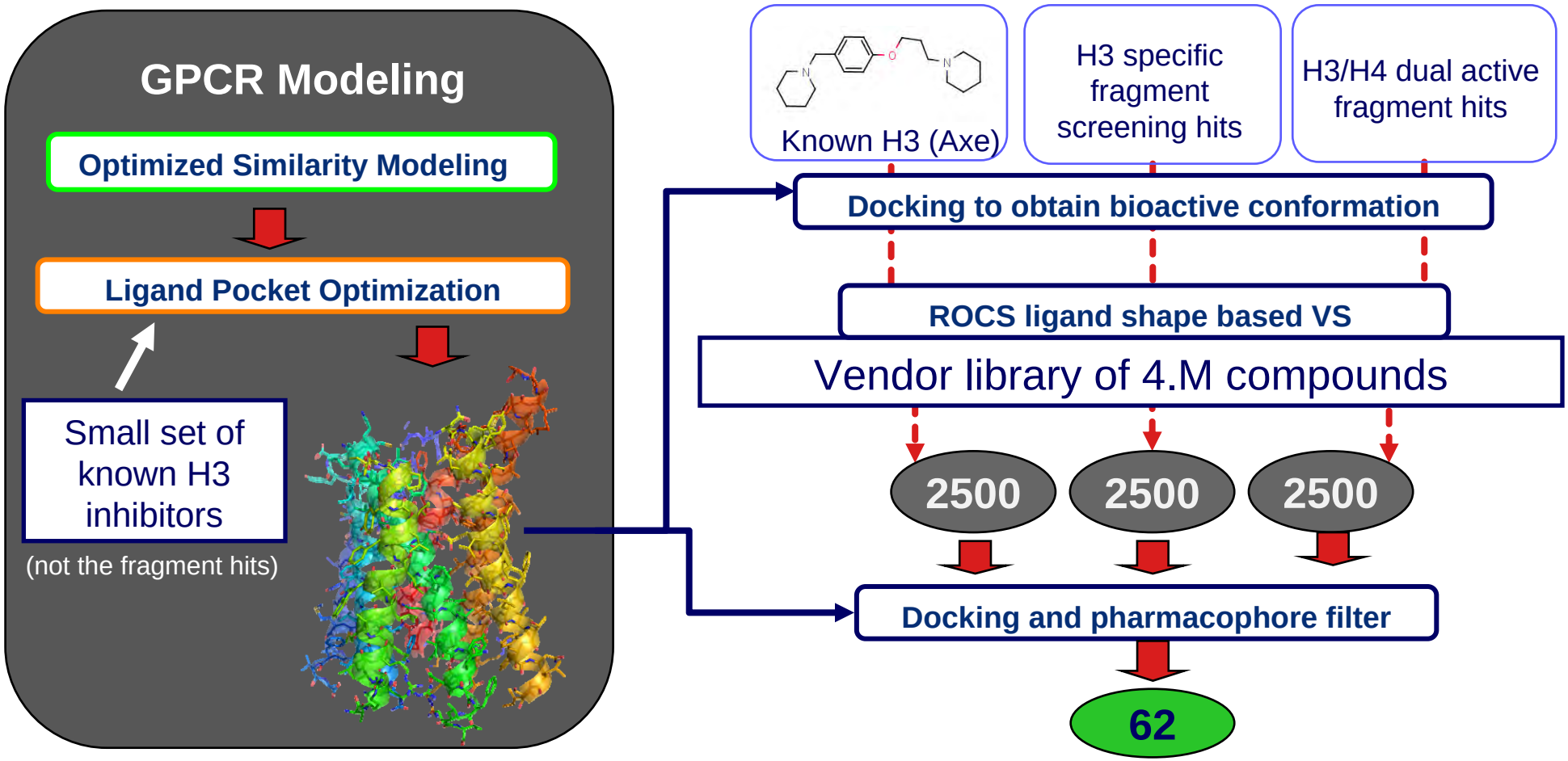
- A random set of 1,700 fragments (out of 20,000) was tested in quadruplicates
- At 2µM and 20µM in functional Ca^{2+} flux assays (then IC_{50}s for actives)
- On cell lines expressing either the histamine receptors H1, H3, or H4 to identify sub-type specific antagonists
- 106 hits id'd from 1ry screen + confirmed
- Of these, 64 H3 selective, 21 H4 selective (>2x) (all >1µM)



- We then sought to expand on these hits using ligand & structure-based VS

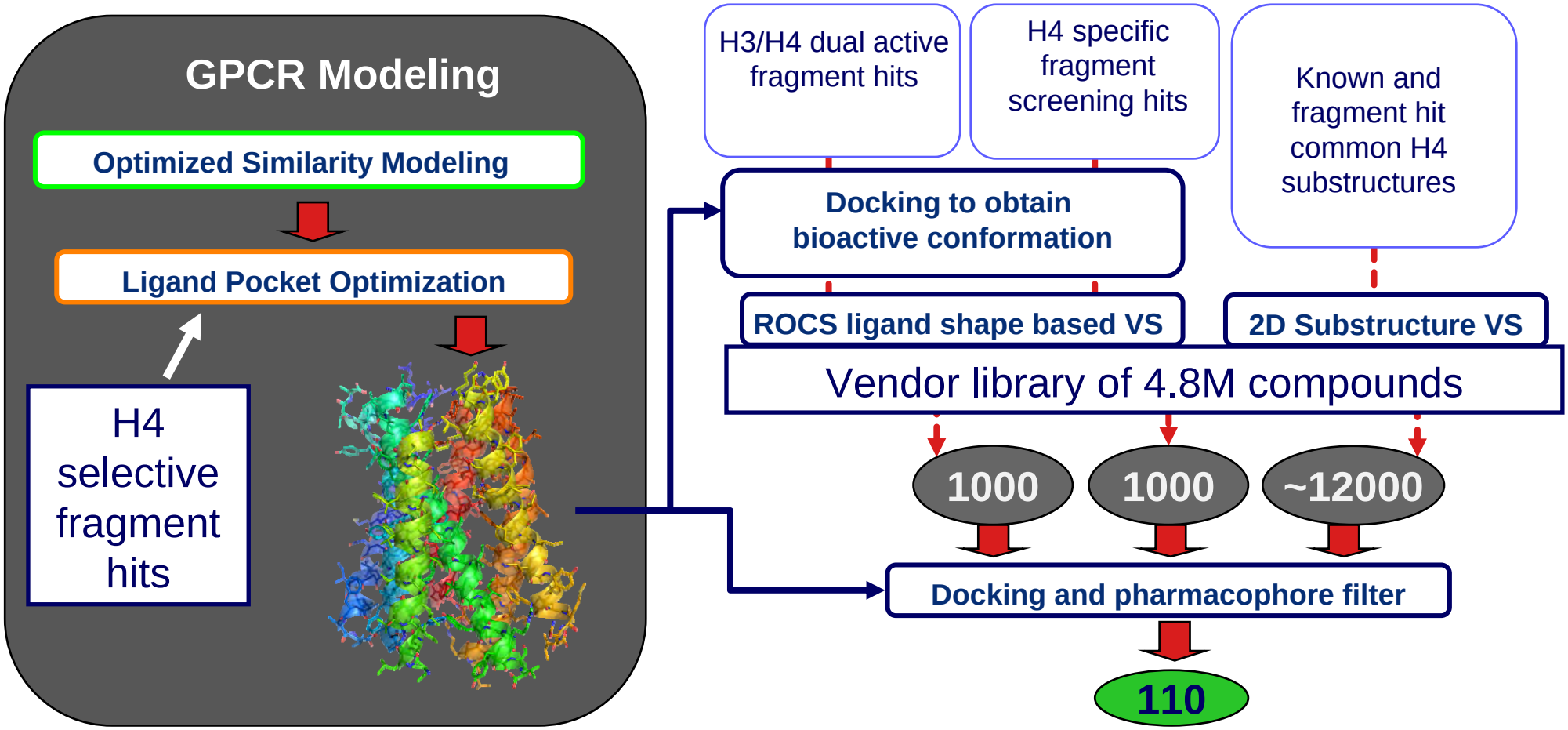
H3 GPCR modelling and virtual screening using fragment data

Schematic of the VS process



H4 GPCR modelling and virtual screening using fragment data

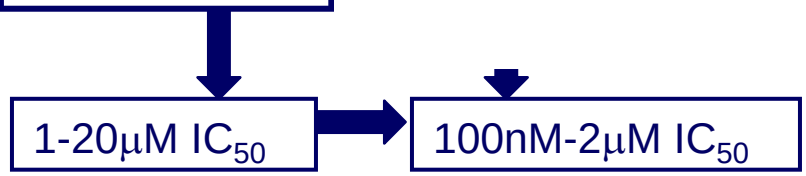
Schematic of the VS process



Enrichment for H3 or H4 antagonists

A few fragment and virtual screening stats

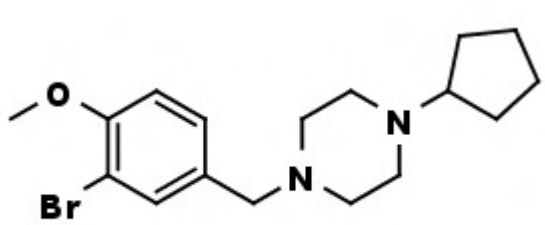
	Fragments
# Compounds screened	1,700
Confirmed hits	106
Hit rate	6%
H3 selective hits	64
H4 selective hits	21



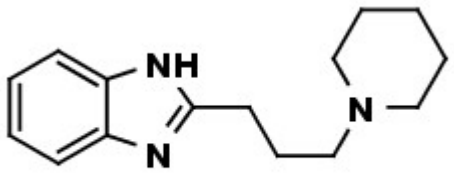
- Combination of ligand and structure-based VS produced very good enrichment
- Successful selection of H3 selective compounds
- Much less good selection of H4 selective compounds

H3 selective compounds

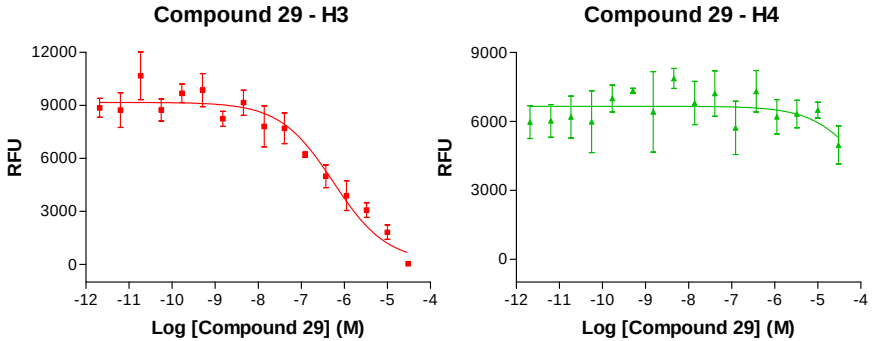
Compounds identified by VS fragment expansion



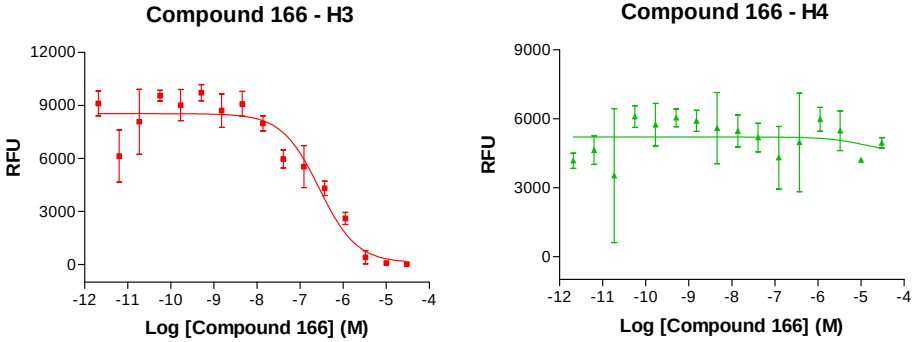
Compound 29



Compound 166



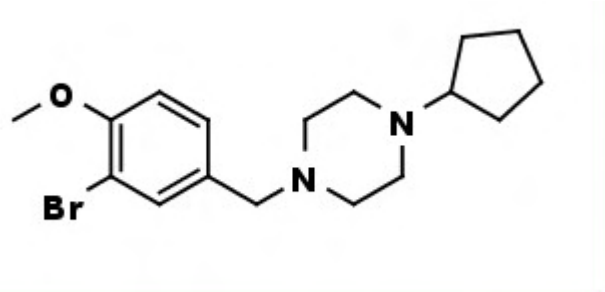
IC_{50} = 556 nM



IC_{50} = 279 nM

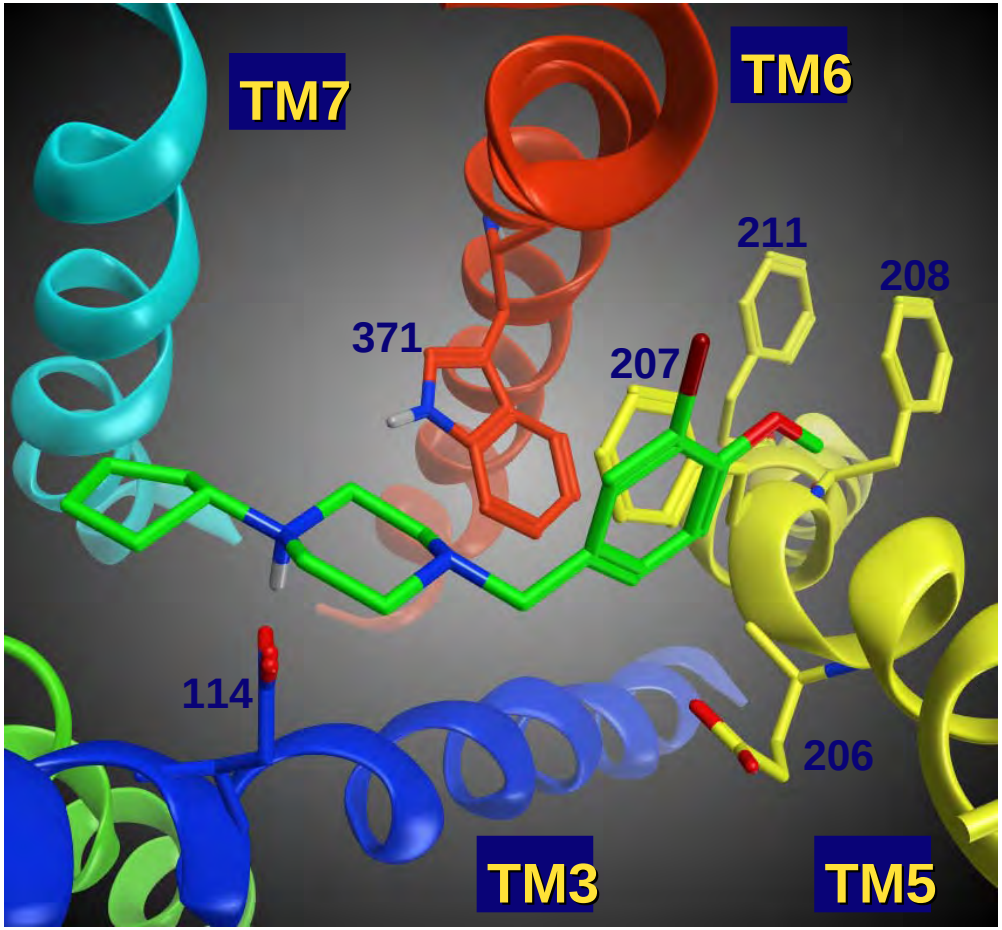
H3 selective compounds

Compounds identified by VS fragment expansion



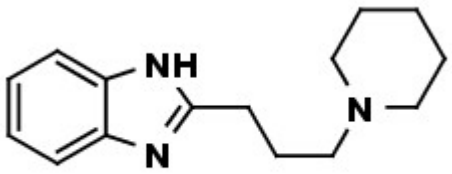
VS hit IC_{50} = 560 nM

- Single basic amine interaction
- Not optimal aromatic interaction position
- Br + M-O not really doing anything



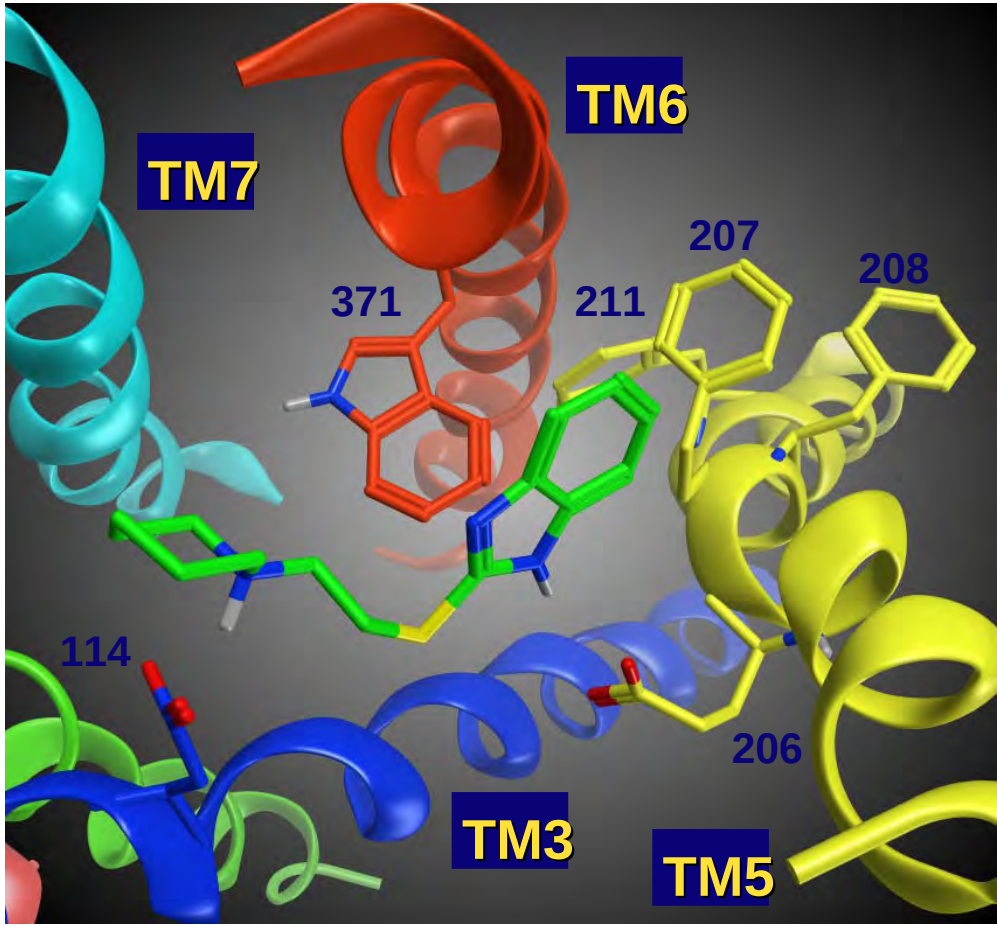
H3 selective compounds

Compounds identified by VS fragment expansion



IC_{50} = 279 nM

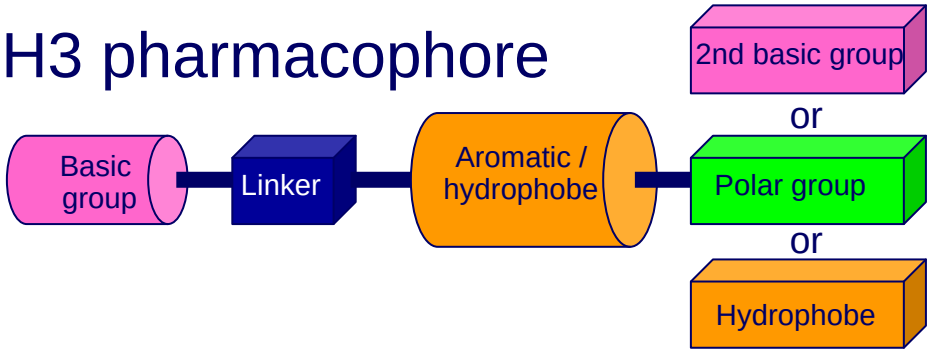
- Two basic amine interactions (D114 & E206) (not necessary)
- Suboptimal aromatic interactions
- Selectivity; H3 are able to accommodate short or long compound (H4 only short) – long compounds with good aromatic interactions key to H3 selectivity



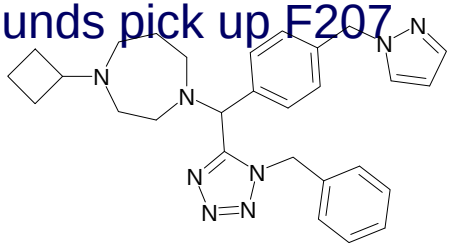
H3 modeling conclusions

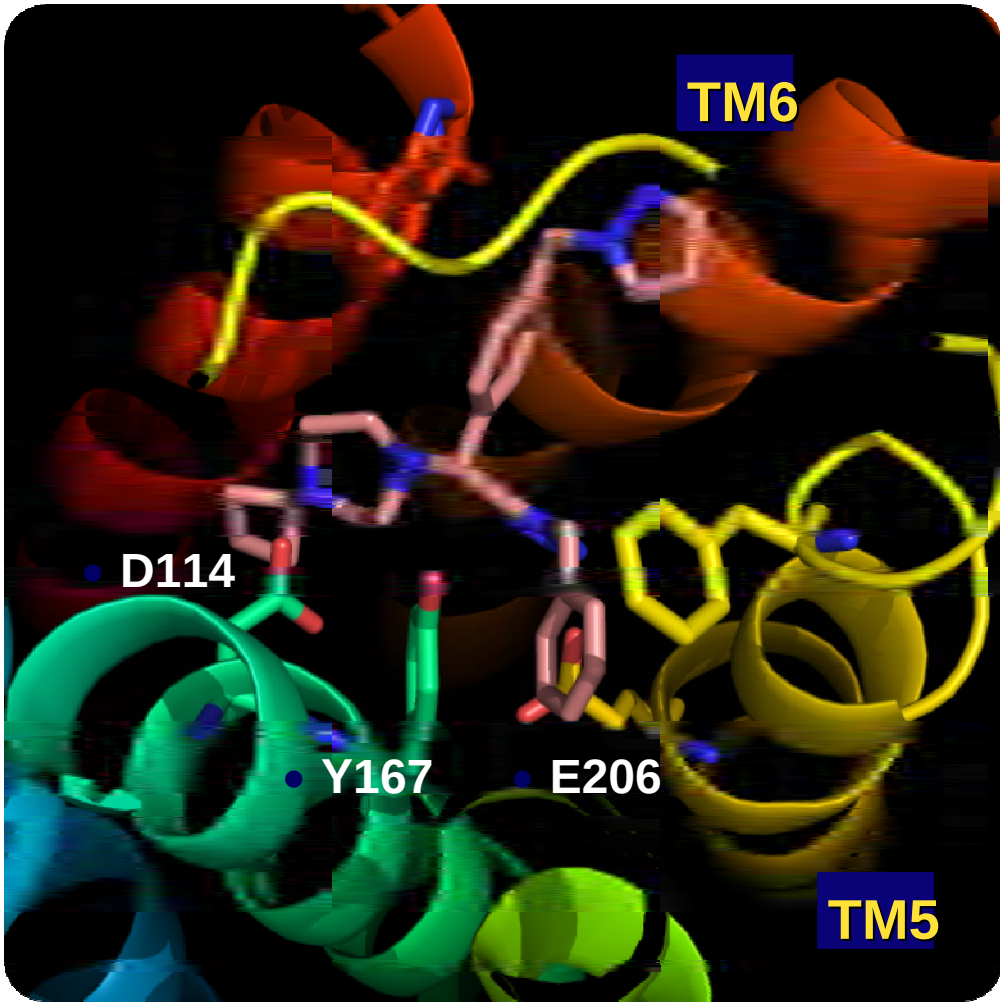
Accommodating single basic centre – long & short pockets

H3 pharmacophore



- Single basic centre to D114 – no need for E206 interaction if others (e.g. Y167) compensate
- H3 is able to accommodate short or long compounds – longer compounds pick up E207

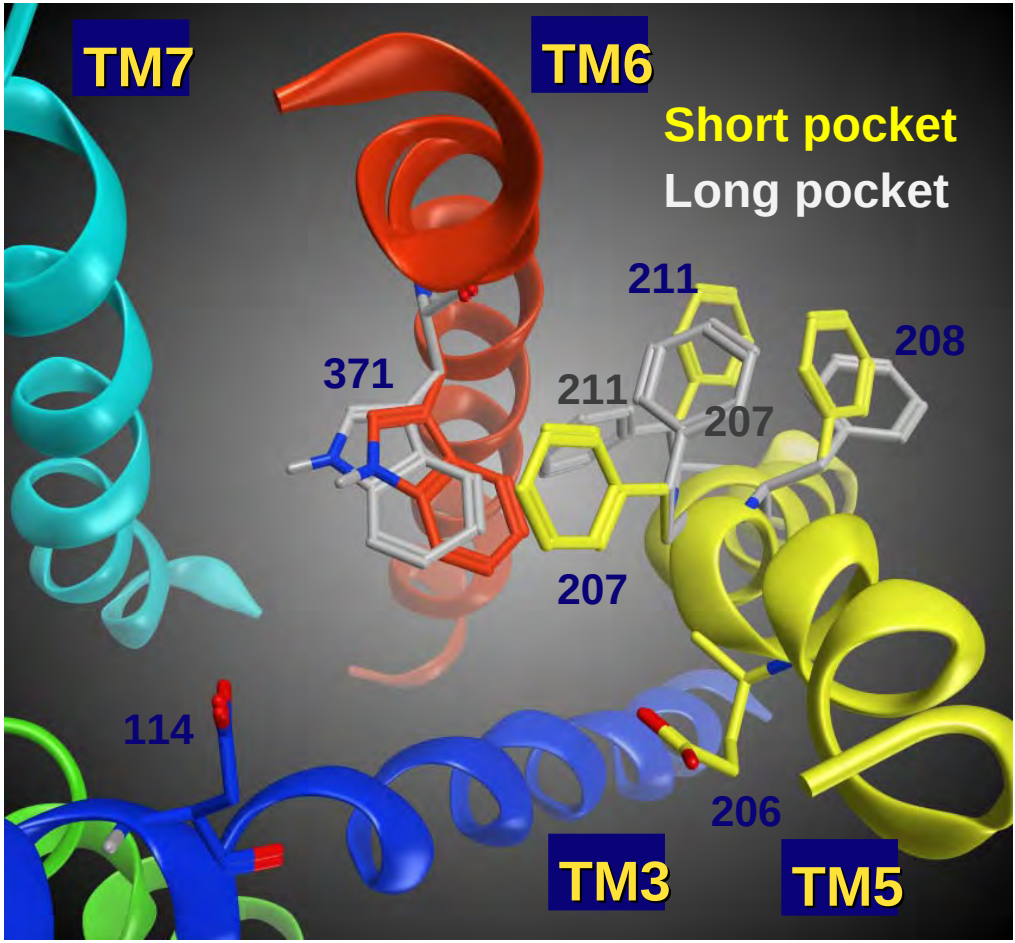




H3 modeling conclusions

Accommodating single basic centre – long & short pockets

- The “extension” of the pocket is achieved by the “switch” between the conformations of Phe207 and Phe211 when interaction between aromatic residues of TM5 and Trp371 (TM6) are maintained
  - **Short pocket:** Trp371 (TM6) interacts with Phe207 (TM5) & Phe208 (TM5) with Phe211 (TM5)
  - **Long pocket:** Trp371 (TM6) interacts with Phe211 (TM5) & Phe207 (TM5) with Phe208 (TM5)



H3 vs H4 pockets

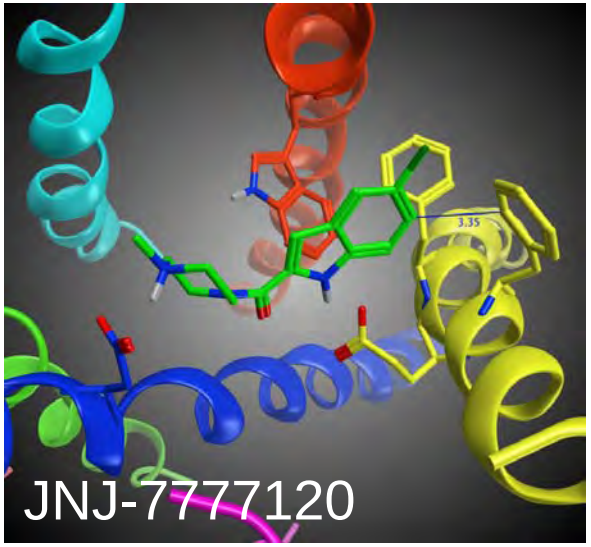
F differences

- The major difference observed in TM5 helix:

```
# Identity:      12/27 (44.4%)
# Similarity:    16/27 (59.3%)


                                    208  211
H3.TM5      NWYFLITASTLEFFTPFLSVTFFNLSI
            .||.|...|.|||..|.:.|.:||::|
H4.TM5      EWYILAITSFLEFVIPVILVAYFNMNI
                              180
```

No switch F res. in H4



- Compared to H3, H4 does not have F208 & F211 and the “switch” mechanism does not exist for H4 (optimized models more easily select a single state for H4)
- As a result of this H4 can accommodate only short sized ligands
- On the other hand, H4 has F180 that H3 doesn't have

Summary

GPCR modeling + fragment screening

- Screening of fragments against GPCRs is a valid approach to identify active compounds for these receptors
- The combined fragment screening and *in-silico* approaches can:
  - Identify neighbours to hit structures to establish a SAR
  - Reduce time and costs to identify potential starting points for medicinal chemistry
  - Accelerate hit-to-lead
- Identified fragment hits can be used as tools to refine existing GPCR models
- In turn the refined GPCR models can be used to drive the next round of compound optimisation
- The staged screening/comp. chem. expansion/design process results in a good enrichment of active compounds

Acknowledgments

GPCR Modeling

- Sandeep Pal
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- Dave Hallett
- Thomas Hesterkamp

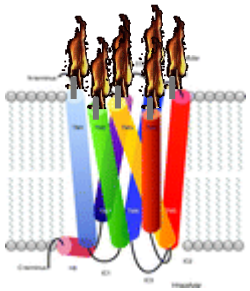
H3 Chemistry

- Adam Davenport

H3 Biology Team

- Mark Slack
- Andreas Kahrs (BI)

- And to me because it's my birthday today!



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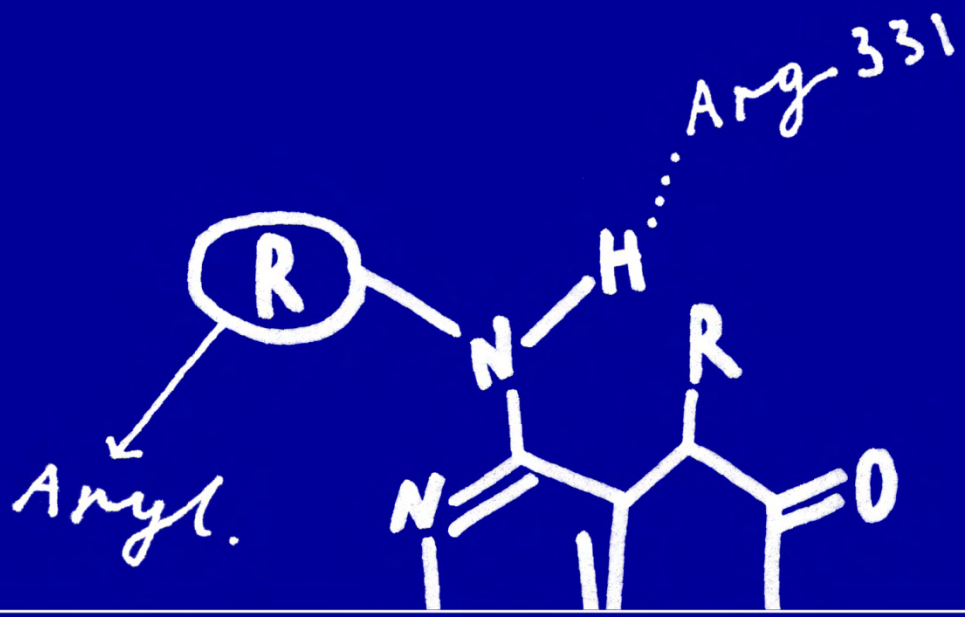
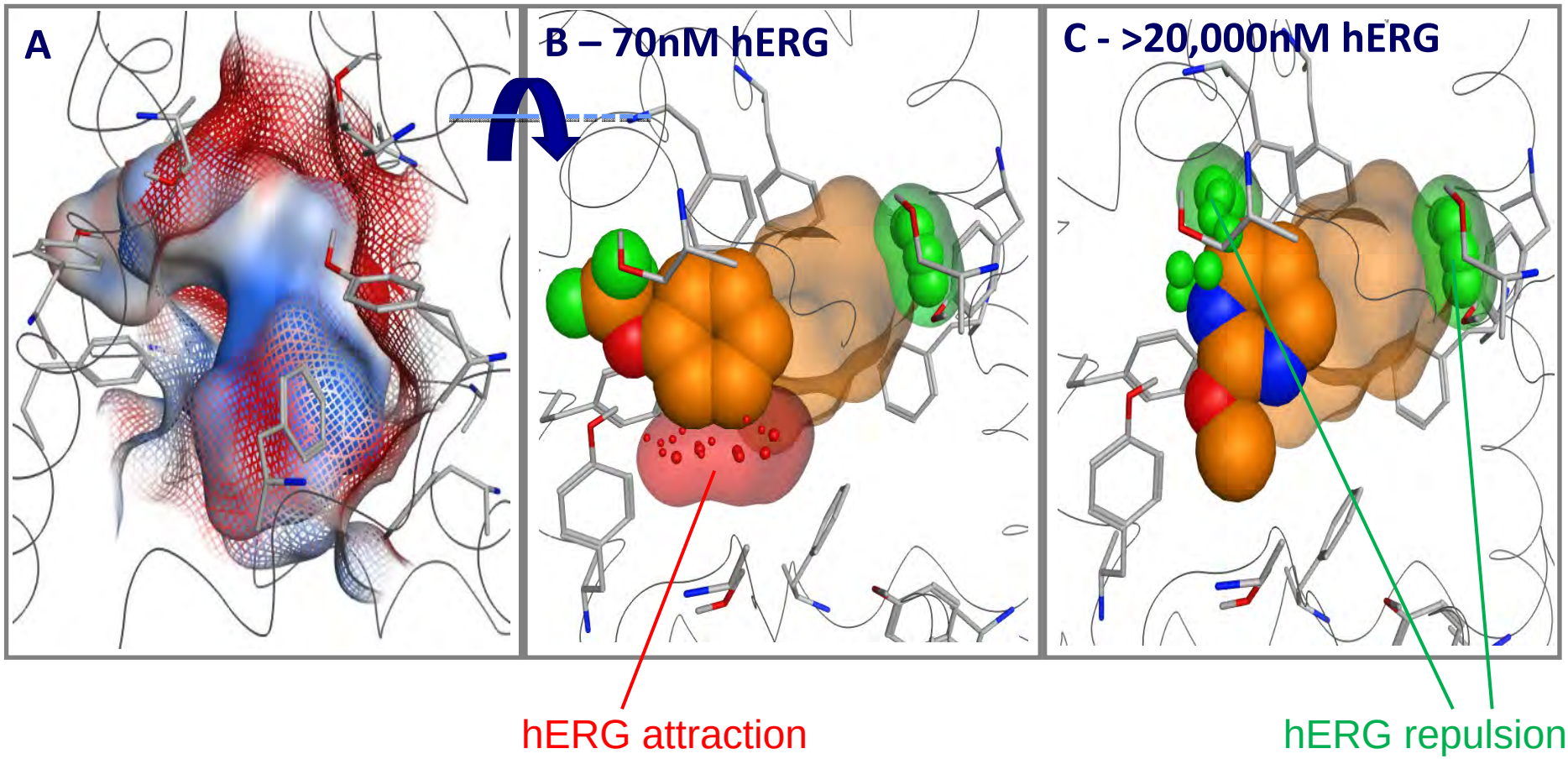


Back Up slides

Abolishing hERG activity for H3 compounds

Use electrostatic complementarity hERG modeling



Alchemia VAST + Evotec GPCR Modelling of MCH-1

Alchemia compounds used in ligand pocket optimization stage of modelling

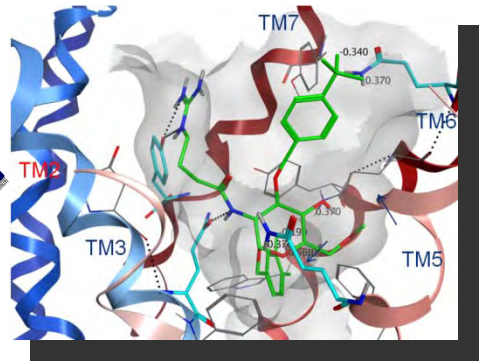
Homology Modeling based on single template

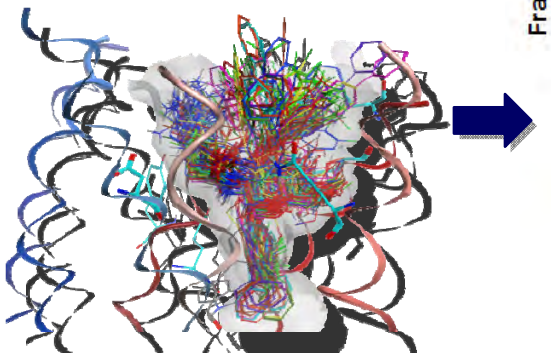
Hybridized Homology Modeling

Optimized Similarity Modeling

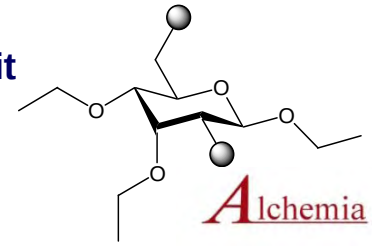
Ab-initio Decoy Template Modeling

Ligand Pocket Optimization

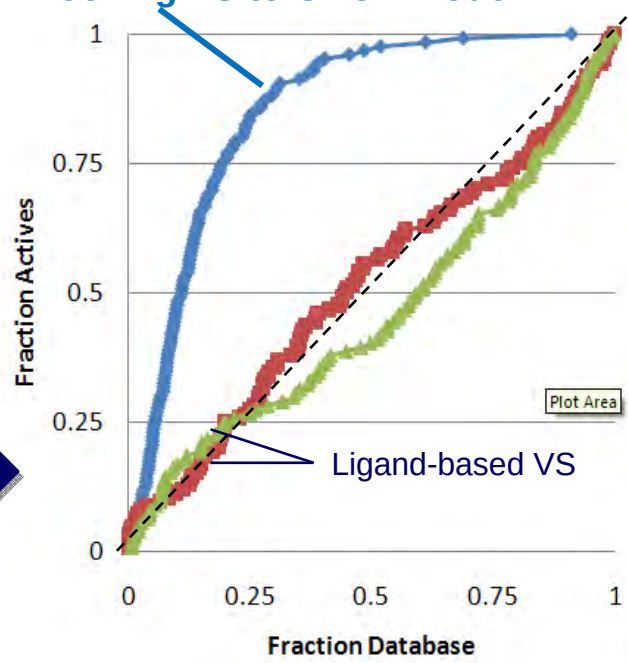




Alchemia's VAST technology involves hit finding for GPCRs using a library of derivatized amino-pyranose "sugar" scaffold compounds



Docking VS to GPCR Model



- The Alchemia compounds are ideal for exposing the chemical features of compounds required to hit GPCR targets due to their size, complexity and core scaffold rigidity
- Particularly useful for chemokine and orphan GPCRs